Crystal structures of two tropinone reductases: Different reaction stereospecificities in the same protein fold. (MOLECULAR BIOFUNCTION-Functional Molecular Conversion)

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Crystal structures of two tropinone reductases: Different reaction stereospecificities in the same protein fold.

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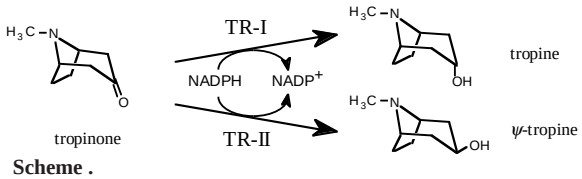
A pair of tropinone reductases (TRs) share 64% identical amino acid residues, and belong to the short-chain dehydrogenase/reductase family. In the synthesis of tropane alkaloids in several medicinal plants, the TRs reduce a carbonyl group of an alkaloid intermediate, tropinone, to hydroxy groups having different diastereomeric configurations. To clarify the structural basis for their different reaction stereospecificities, we determined the crystal structures of the two enzymes at 2.4- and 2.3-Å resolutions. The overall folding of the two enzymes was almost identical. The substrate binding site was composed mostly of hydrophobic amino acids in both TRs, but the presence of different charged residues conferred different electrostatic environments on the two enzymes.

Keywords : X-ray crystallography/ Stereospecificity/ Enzymatic reaction/ alkaloids/ Tropinone reductase/ NADPH

Two tropinone reductases (TRs) constitute a branching point in the biosynthetic pathway of tropane alkaloids, which include such medicinally important compounds as hyoscyamine (atropine) and cocaine. TRs catalyze NADPH-dependent reductions of the 3-carbonyl group of their common substrate, tropinone, to hydroxy groups with different diastereomeric configurations: TR-I (EC 1.1.1.206) produces tropine (3α-hydroxytropane), and TR-II (EC 1.1.1.236) produces pseudotropine (ψ-tropine, 3β-hydroxytropane) (**Scheme**). The most intriguing question concerning the two TRs is what protein structures enable the enzymes to produce different stereoisomers from the same substrate, tropinone. Only a small number of the amino acid residues that differ between TR-I and TR-II may actually participate in determining the stereospecificities, and the overall foldings of the two enzymes may not be as different as predicted from their primary structures (1). To verify this idea, we determined the crystal structures of the TRs from *Datura. stramonium* (2, 3). The structures revealed a simple evolutionary process adopted by the TRs to acquire their different stereospecificities.



Scheme .

MOLECULAR BIOFUNCTION — Functional Molecular Conversion —

Scope of research

Our research aims are to elucidate structure-function relationships of various biocatalysts in combination with organic chemistry, molecular biology and X-ray crystallography, and to clarify real physiological roles in tea plants of a glycosidase and β-primeverosidase, the latter which was found by ourselves to be mainly concerned with aroma formation during tea manufacturing. Main subjects are (1) Design and synthesis of transition-state analogue inhibitors of ATP-dependent ligases, (2) Chemical, biochemical and molecular biological studies on primeverosidase, (3) Time-resolved X-ray crystallographic study of glutathione synthetase, (5) Development of a new type of microbial lipase by evolutionary molecular engineering, (6) X-Ray crystallography of wild-type and mutant firefly luciferases, and (7) Overexpression and purification of pyruvate phosphate dikinase from Maize.





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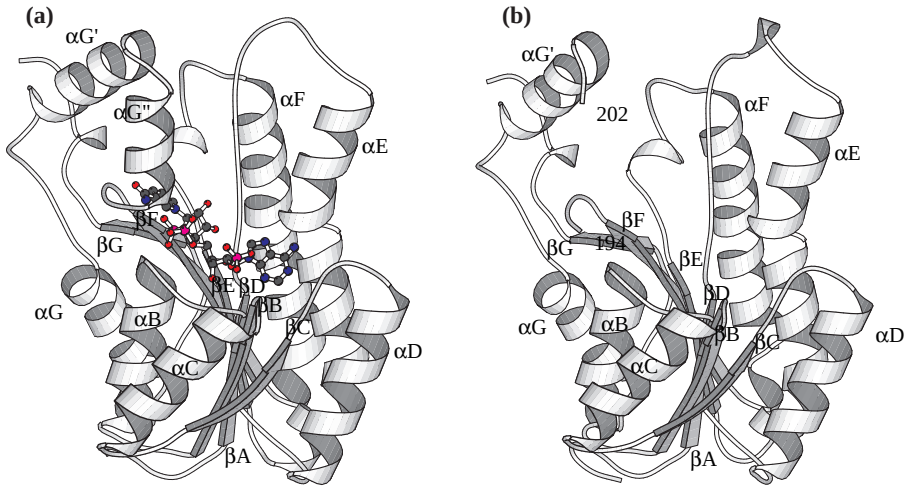

Figure 1.
Subunit structure of TR-I (a), and TR-II (b).

The subunit structures of the TR-I and TR-II dimers are shown in Fig. 1. The two structures are almost indistinguishable from each other in both subunit folding. Conservation of the subunit structures between TR-I and TR-II was substantiated when the two structures were superimposed by the least squares method using all equivalent Cα positions (rms deviation = 0.78 Å). Both TR subunits consist of a core domain that includes most of the polypeptide and a small lobe that protrudes from the core. A deep cleft was recognized between the core domain and the small lobe, which is presumed to be the binding site for tropinone. In the center of the core domain is a seven-stranded parallel β-sheet, flanked on each side by three α-helices, which constitutes the 'Rossmann fold' topology. This core structure is highly conserved among the SDR family members, despite relatively low residue identity between these enzymes (~30%). The small lobes of the two TRs are also very similar to each other, although the structure of this region is highly variable among SDRs for which crystal structures are known. In TR-II, the polypeptide corresponding to αG" is disordered, and therefore could not be modeled.

TR-I protein was crystallized in the presence of $NADP^+$, and the bound cofactor molecules in the protein structure could be modeled unambiguously. As seen in Fig. 1a, $NADP^+$ is located at the bottom of the cleft between the core domain and the small lobe.

Concurrent conservation of the catalytic residues and the cofactor-binding sites leaves only one explanation for the TR stereospecificities; tropinone should bind TR-I and TR-II in opposite orientations. The bound tropinone was predicted to contact several amino acids in both TRs (Fig. 2). These residues are located either at the two loops in the core domain or in the two a-helices that constitute the small lobe. The positive charge on the TR-I surface is due to His112, which in TR-II is replaced by Tyr100, a polar but not basic residue. The negative charge on the TR-II surface is generated by Glu156,

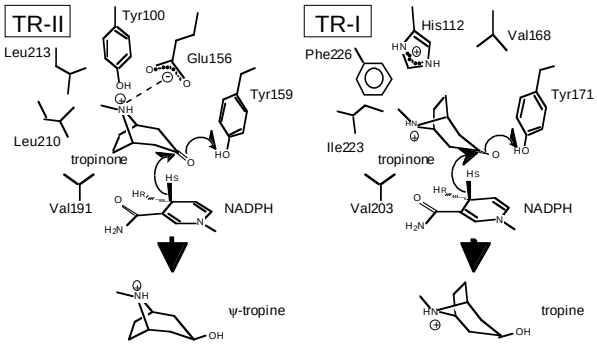

Figure 2. Schemetic view of the predicted active sites of TR-I and TR-II.

which is replaced by the hydrophobic Val168 in TR-I. As the nitrogen atom of tropinone is positively charged under physiological pH conditions, the charge distributions in the tropinone-binding sites agreed well with the predicted orientations of tropinone. In contrast, TR-I uses a novel means to orient tropinone, namely repulsion between the positive charges of His112 and the nitrogen atom of tropinone. Apart from the charged residues described above, most of the amino acids that would contact tropinone within the binding sites, are hydrophobic. These residues would provide a favorable environment for the binding of tropinone which generally has a hydrophobic nature.

The structures presented here are the first for a pair of enzymes that are closely related evolutionarily but which have different reaction stereospecificities. Comparison of the two TR structures made clear that opposite reaction stereospecificities can be acquired in enzymes that have a conserved overall folding, by changing the amino acids in the substrate-binding site.

References

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